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Anticonvulsant Activity of Hydroalcoholic Extract of *Citrullus colocynthis* Fruit: Involvement of Benzodiazepine and Opioid Receptors

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Abstract

This study investigated the anticonvulsant activity *of Citrullus colocynthis* fruit extract used traditionally in the treatment of convulsion. Albino mice were pretreated with extract in different doses (10, 25, 50, and 100 mg/kg), prior to injection of pentylenetetrazole. Animals received pretreatments with naloxone and flumazenil to further clarify the mechanisms of anticonvulsant action. The total flavonoid content of *Citrullus colocynthis* extract was also determined. *Citrullus colocynthis* hydroalcoholic extract with doses 25 and 50 mg/kg prolonged the onset of seizures and decreased the duration compared with control group. Pretreatment by flumazenil could inhibit the effect of *Citrullus colocynthis* on latency of seizure to some extent and administration of naloxone significantly inhibited changes in latency and duration of seizure produced by *Citrullus colocynthis*. This study showed that *Citrullus colocynthis* has significant anticonvulsant effect in pentylenetetrazole-induced seizures in mice, and these effects may be related to its effect on γ-aminobutyric acid-ergic and opioid systems. These results confirmed the traditional use of *Citrullus colocynthis* in Iranian traditional medicine.

Keywords

Citrullus colocynthis, anticonvulsant, naloxane, pentylenetetrazole, traditional persian medicine

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Epilepsy is one of the most common neurological disorders that affects about 50 million people worldwide.[1] There is an increasing need to explore new natural medicines due to difficulties of classic treatments, that is, inefficiency or side effects and chronic toxicity of drugs.[2] So, there is an increasing interest to use natural remedies that could be effective alternatives. There are some herbs in Iranian traditional medicine for treatment of epilepsy.[3] *Citrullus colocynthis* (L.) Schrad. (Cucurbitaceae), Known as bitter apple or wild gourd, is one of the medicinal plants recommended for treatment of seizure.[4] Other pharmacological activities have been also mentioned in Iranian traditional medicine such as purgative, anti-inflammatory, antidiabetic, analgesic, hypolipidemic, antioxidant, abortifacient, and antiepileptic.[4,5]

This plant grows in south, center, and east of Iran. The main medicinal part of the plant is the fruit pulp, and it is better to use the fruit pulp for extraction. So far, different biological activities were reported from *Citrullus colocynthis*.[4] Fruits of *Citrullus colocynthis* showed analgesic, purgative, anti-inflammatory, antioxidant, antidiabetic, and hypolipidemic activities.[6-11] Several bioactive compounds of *Citrullus colocynthis* fruit have been reported in the literature. They are grouped as glycosides, flavonoids, alkaloids, carbohydrates, fatty acids, and essential oils.[12-14] Cucurbitacins have been reported as the main chemical constituents of *Citrullus colocynthis* fruits.

According to our investigations, the antiepileptic effect of *Citrullus colocynthis* has not been studied so far. Following our previous studies on Iranian medicinal plant,[3] in the present study, anticonvulsant activity and possible mechanisms of action of *Citrullus colocynthis* extract were studied in pentylenetetrazole-induced seizures in mice for the first time.

Methods

Plants Material

Citrullus colocynthis fruits were purchased from a local herbal market in Tehran, Iran and its authenticity was approved by Dr G. Amin, member of the School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (No. PmP-640). The seeds were

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removed from fruit pulps and the pulps were dried. Hydroalcoholic extract (70:30 alcohol:water) of fruit pulps was prepared by maceration method (3 times) and the extract was dried by rotary evaporator. The extract was dissolved in normal saline when used in the animal study.

Total Flavonoid Content

The total flavonoid content of *Citrullus colocynthis* extract was determined using $AlCl_3$ reagent.[15] Briefly, 2.5 mL of each sample (and/or quercetin as the standard), previously dissolved in 90% ethanol, was mixed with 2.5 mL of a 2% $AlCl_3$ solution in 90% ethanol. After 40 minutes, the absorbance of the yellow color produced was measured at 425 nm. The total flavonoid content (as mmicrograms [µg] quercetin equivalents/milligram [mg] of sample) for the sample was calculated on the basis of a linear calibration curve obtained using quercetin ($y = 0.0169x + 0.3526$, $r^2 = 0.995$).

Animals

Male albino mice (n = 36, weight 25-30 g) were housed in animal unit of Iran University of Medical Sciences under standard laboratory conditions (temperature 23°C ± 2°C) with 12-hour dark and 12-hour light cycle. The animals were fed with standard dry pellet diet and tap water ad libitum. All possible steps were taken to avoid animal suffering at each stage of the experiment.

Ethics

This study was performed according to the guidelines of the US National Institutes of Health (NIH publication no. 85.23, revised 1985) guides for the care of lab animals.

Chemicals

Pentylenetetrazole (Sigma, St Louis, MO, USA) and diazepam (Darupakhsh, Iran), naloxone (Sigma) and flumazenil (Darupakhsh, Iran) were purchased from commercial sources.

Experimental Studies

Anticonvulsant Activity. Seizures were induced in mice with standard agent, pentylenetetrazole (60 mg/kg, intraperitoneally).[16,17] The animals were divided randomly into 6 groups (n = 6). Group I served as a control and received normal saline (10 mL/kg, intraperitoneally), group II was treated with diazepam (1 mg/kg, intraperitoneally), as positive control group and groups III to VI, respectively, received the extract in doses of 10, 25, 50, and100 mg/kg, intraperitoneally. After 30 minutes, all groups received the chemoconvulsant agent, pentylenetetrazole. Following induction of convulsions in mice with intraperitoneal injections of pentylenetetrazole, the animals were observed for 30 minutes for signs of neurological deficits, particularly hindlimb tonic convulsions. Hindlimb tonic extensions of the mice were regarded as seizures. Animals without any convulsion after 30 minutes were considered as protected. The latency and duration of seizures were recorded for 30 minutes after injection of pentylenetetrazole in the unprotected animals. Also, the animals were observed for mortality for 24 hours after pentylenetetrazole administration.

Evaluation of the Effect of Flumazenil on the Anticonvulsant Activity of Citrullus colocynthis *Extract.* The effect of flumazenil, a benzodiazepine receptor antagonist, on the anticonvulsant activity of *Citrullus colocynthis* was studied in order to investigate the probable involvement of benzodiazepine receptors.[18,19] Animals were divided randomly into 6 groups (n = 6). In the first group, mice were given flumazenil (2 mg/kg) 5 minutes before the administration of *Citrullus colocynthis* (50 mg/kg, intraperitoneally) and 35 minutes before the injection of pentylenetetrazole. In the second group, the animals received flumazenil (2 mg/kg) 5 minutes before the administration of diazepam (1 mg/kg). Also, 3 groups were injected diazepam (1 mg/kg, intraperitoneally), flumazenil (2 mg/kg), and normal saline 30 minutes before the administration of pentylenetetrazole (60 mg/kg, intraperitoneally) respectively.[18,19] Then latency and duration of convulsion in 30 minutes after injection of pentylenetetrazole, protection percentage and also mortality rate during the first 24 hours were recorded. The anticonvulsant activity of *Citrullus colocynthis* and diazepam in mice pretreated with flumazenil was assessed and compared with normal saline (10 mL/kg), flumazenil (2 mg/kg), diazepam (1 mg/kg), and *Citrullus colocynthis* (50 mg/kg, intraperitoneally) treated animals.

Evaluation of the Effect of Naloxone on the Anticonvulsant Activity of Citrullus colocynthis *Extract.* Animals were divided to 4 groups randomly (n = 6) for further investigation of probable modulatory effect of opioid receptors on the anticonvulsant activity of *Citrullus colocynthis*.[19,20] Naloxone, an opioid receptor antagonist, was administered (5 mg/kg, intraperitoneally) 5 minutes before *Citrullus colocynthis* (50 mg/kg, intraperitoneally) and 35 minutes before the injection of pentylenetetrazole to animals.[20,21] The anticonvulsant activity of *Citrullus colocynthis* in groups pretreated with naloxone was compared with animals pretreated only with *Citrullus colocynthis* (50 mg/kg, intraperitoneally), naloxone (5 mg/kg), and normal saline (10 mL/kg).

Statistical Analysis

The results are reported as mean ± standard error of the mean and tested with 1-way analysis of variance followed by the multiple comparison test of Tukey-Kramer. Results with $P < .05$ were considered as statistically significant.

Results

Total Flavonoid Content

Total flavonoid content of the *Citrullus colocynthis* hydroalcoholic extract as micrograms quercetin equivalents/milligram of sample was 10.7 ± 0.1 mg/g (mean ± standard error of the mean, n = 3).

Anticonvulsant Activity on Pentylenetetrazole-Induced Seizure

Injection of the *Citrullus colocynthis* extract (25 and 50 mg/kg) prolonged significantly the onset of seizure ($P < .05$ and $P < .01$, respectively) and decreased the duration of seizures ($P < .01$) compared with the saline group. Also, *Citrullus colocynthis* extract (10 and 100 mg/kg) decreased duration of seizures significantly ($P < .05$), but had no significant effect on

Table 1. Effect of *Citrullus colocynthis* Extract on Pentylenetetrazole-Induced Seizure in Mice.[a]

Treatment Group	% Protection (30 min)	Seizure Latency (s)	Seizure Duration (s)	Mortality (24 h)
PTZ + NS (10 mL/kg)	0	83.75 ± 12.36	31.60 ± 3.58	4/6
PTZ + diazepam (1 mg/kg)	100	—	—	0/6
PTZ + *Citrullus colocynthis* (10 mg/kg)	0	210.7 ± 36.40	18.34 ± 5.06*	2/6
PTZ + *Citrullus colocynthis* (25 mg/kg)	16.66	727.7 ± 242.0*	12.28 ± 3.55**	1/6
PTZ + *Citrullus colocynthis* (50 mg/kg)	16.66	912.0 ± 205.2**	11.95 ± 3.48**	0/6
PTZ + *Citrullus colocynthis* (100 mg/kg)	0	320.4 ± 60.45	17.51 ± 3.85*	2/6

Abbrevations: NS, normal saline; PTZ, pentylenetetrazole.
[a]Data are expressed as mean ± standard error of the mean (n = 6).
*$P < .05$, **$P < .01$ compared with the control group.

Table 2. Effect of Flumazenil on the Anticonvulsant Activity of *Citrullus colocynthis* Extract in Pentylenetetrazole-Induced Convulsion in Mice.[a]

Treatment Group	% Protection (30 min)	Seizure Latency (s)	Seizure Duration (s)	Mortality (24 h)
PTZ + NS (10 mL/kg)	0	83.75 ± 12.36	31.60 ± 3.58	4/6
Flumazenil (2 mg/kg)	0	80.50 ± 5.23	25.83 ± 2.12	3/6
Diazepam (1 mg/kg)	100	—	—	0/6
Diazepam + flumazenil	0	116 ± 10.65	29.33 ± 2.28	2/6
Citrullus colocynthis (50 mg/kg)	16.66	912.0 ± 205.2***	11.95 ± 3.48***	0/6
Citrullus colocynthis (50 mg/kg) + Flumazenil	0	294.4 ± 75.8**	10.00 ± 3.06***	3/6

Abbrevations: NS, normal saline; PTZ, pentylenetetrazole.
[a]Data are expressed as mean ± standard error of the mean (n = 6).
**$P < .01$ compared with the *Citrullus colocynthis* (50 mg/kg) group. ***$P < .001$ compared with the control group.

Table 3. Effect of Naloxone on the Anticonvulsant Activity of *Citrullus colocynthis* in Pentylenetetrazole-Induced Convulsion in Mice.[a]

Treatment Group	% Protection (30 min)	Seizure Latency (s)	Seizure Duration (s)	Mortality (24 h)
PTZ + NS (10 mL/kg)	0	83.75 ± 12.36	31.60 ± 3.58	4/6
Naloxone (5 mg/kg)	0	50.33 ± 4.34	48.50 ± 3.88	4/6
Citrullus colocynthis (50 mg/kg)	16.66	912.0 ± 205.2***	11.95 ± 3.48*	0/6
Citrullus colocynthis+ naloxone	0	129.9 ± 28.26**	29.33 ± 11.10	3/6

Abbrevations: NS, normal saline; PTZ, pentylenetetrazole.
[a]Data are expressed as mean ± standard error of the mean (n = 6).
*$P < .005$, ***$P < .001$ compared with the control group. **$P < .01$ compared with the *Citrullus colocynthis* (50 mg/kg) group.

latency. In addition, *Citrullus colocynthis* extract (25 and 50 mg/kg) exhibited protection against seizure in 16.66% of animals and reduced mortality compared with the control group. Diazepam (1 mg/kg) protected 100% of mice from pentylenetetrazole-induced seizure and mortality (Table 1).

The Effect of Flumazenil on the Anticonvulsant Activity of Citrullus colocynthis *Extract*

Administration of flumazenil (2 mg/kg) 5 minutes before *Citrullus colocynthis* hydroalcoholic extract (50 mg/kg) and 35 minutes before the injection of pentylenetetrazole, resulted in inhibition of prolonged latency produced by extract solution ($P < .01$); but had no significant effect on duration of seizure. So, flumazenil reversed the action of *Citrullus colocynthis* extract to some extent. Also, flumazenil could reverse the anticonvulsant activity of diazepam (Table 2).

The Effect of Naloxone on the Anticonvulsant Activity of Citrullus colocynthis *Extract*

In the group receiving naloxone (5 mg/kg) before *Citrullus colocynthis* extract (50 mg/kg) and pentylenetetrazole, the effect of *Citrullus colocynthis* extract was reversed in prolonging seizure latency ($P < .01$) and duration of seizure compared with the group treated with *Citrullus colocynthis* extract (50 mg/kg; Table 3).

Discussion

According to complexity in treatment of epilepsy with modern antiepileptic drugs, interest in discovering new alternative medicine from natural sources has increased.[2] In Iranian traditional medicine, some herbs, such as *Citrullus colocynthis*, have been recommended for treatment of epilepsy.[4]

Citrullus colocynthis is used in different parts of the world to treat different diseases, including diabetes, some gastrointestinal disorders, leprosy, asthma, bronchitis, jaundice, joint pain, cancer, and mastitis as well as common cold, cough, toothache, and wounds.[4,22-24]

Although *Citrullus colocynthis* fruit has been used for many years, reports on systematic toxicity and safety evaluation are rare. Investigating the acute toxicity and histopathological effects of saponin (extracted from whole plant of *Citrullus colocynthis*) on mice, Diwan et al[25] reported the median lethal dose to be 200 mg/kg.

Some traditional use of *Citrullus colocynthis* was confirmed in modern scientific studies. But antiepileptic effect of the fruit pulp of *Citrullus colocynthis* has not been studied so far. So, in this study, anticonvulsant activity and possible mechanisms of action of *Citrullus colocynthis* were studied in an animal model for the first time.

The results of this study indicated that *Citrullus colocynthis* pulp extract demonstrated a statistically significant reduction in the seizures duration and increase in latency period of seizures induced by pentylenetetrazole in mice. This effect increased dose dependently at doses of 10, 25, and 50mg/kg, but decreased at the dose of 100 mg/kg, which may be due to its toxicity.

The main action of the pentylenetetrazole-induced seizure is reducing γ-aminobutyric acid (GABA) level in the cortex. GABA has been reported as the predominant inhibitory neurotransmitter in the central nervous system of mammals and has been implicated in convulsions as it mediates the inhibition of neuronal responsiveness and activity by increasing the chloride-ion conductance through the opening of the chloride-ion channel.[16,26] The findings of the present study tend to suggest that *Citrullus colocynthis* pulp extract might have delayed the occurrence of pentylenetetrazole-induced seizure and reduced its duration by acting on GABAergic system.

Naloxone decreased the prolongation of seizure latency induced by *Citrullus colocynthis* pulp extract and it also antagonized the effect of *Citrullus colocynthis* pulp extract on decreasing the duration of seizures in the pentylenetetrazole model. So, the anticonvulsant effect of *Citrullus colocynthis* is blocked by an antagonist of opioid receptor, and therefore this effect of *Citrullus colocynthis* seems to be related to opioid receptor activation.

Administration of flumazenil before *Citrullus colocynthis* led to decrease in latency of seizures in some extent compared with *Citrullus colocynthis* group. This shows that flumazenil antagonizes the effect of *Citrullus colocynthis* on latency time seizures in the pentylenetetrazole model, so anticonvulsant effect of *Citrullus colocynthis* is probably due to activation of benzodiazepine receptors to some extent. Since opioid and benzodiazepines receptors both are present on GABA receptor complex, it can be deduced that the effect of *Citrullus colocynthis* might be on different receptors modulating the activity of GABA receptor complex. In a study by Meena and Patni,[27] flavonoid quercetin was isolated from *Citrullus colocynthis* and in this study total flavonoid content of *Citrullus colocynthus* extract has been determined according to quercetin. Since flavonoids are the most potent compounds in plants, it is possible that antiepileptic effect of *Citrullus colocynthis* is due to its flavonoid components. Although further studies are necessary to confirm this claim.

In conclusion, the results obtained from this work suggest that *Citrullus colocynthis* pulp extract has anticonvulsant activity and this supports the use of the plant traditionally in the treatment of convulsion. This observed activity of the plant extract on the pentylenetetrazole-induced seizures supports the involvement of the GABA pathway. Further investigation is required to find out which compounds may be responsible for the observed activity and determine their specific mechanism of action.

Author Contributions

The work presented in this article was carried out through collaboration between all authors. MM and AS made the initial hypothesis. All authors participated in defining the research theme and providing the proposal. SM and SAP perform the experiments, collected the data, analyzed the data, and wrote the article. MM and AS conceptualized the study, critically analyzed and discussed the data, and corrected and reviewed the article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

This study was performed according to the guidelines of the US National Institute of Health (NIH Publication no. 85.23, revised 1985) guides for the care of laboratory animals.

References

1. Moshi MJ, Kagashe GA, Mbwambo ZH. Plants used to treat epilepsy by Tanzanian traditional healers. *J Ethnopharmacol*. 2005; 97:327-336.
2. Engel J Jr. Surgery for seizures. *N Engl J Med*. 1996;334:647-653.
3. Abdollahi Fard M, Shojaii A. Efficacy of Iranian traditional medicine in the treatment of epilepsy. *BioMed Res Int*. 2013;2013: 692751.
4. Rahimi R, Amin G, Ardekani MRS. A review on *Citrullus colocynthis* Schrad.: from traditional Iranian medicine to modern phytotherapy. *J Altern Complement Med*. 2012;18:551-554.
5. Khorasani MA. *Makhzan al Advieh*. Tehran, Iran: Bavardaran Press Research institute for Islamic and Complementary Medicine, Iran University of Medical Sciences; 2001.
6. Marzouk B, Marzouk Z, Haloui E, Fenina N, Bouraoui A, Aouni M. Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. *J Ethnopharmacol*. 2010;128:15-19.

7. Marzouk Z, Marzouk B, Mahjoub MA, et al. Screening of the antioxidant and the free radical scavenging potential of Tunisian *Citrullus colocynthis* Schrad. from Mednine. *J Food Agric Environ*. 2010;8:261-265.
8. Nmila R, Gross R, Rchid H, et al. Insulinotropic effect of *Citrullus colocynthis* fruit extracts. *Planta Med*. 2000;66: 418-423.
9. Huseini HF, Darvishzadeh F, Heshmat R, Jafariazar Z, Raza M, Larijani B. The clinical investigation of *Citrullus colocynthis* (L.) schrad fruit in treatment of type II diabetic patients: a randomized, double blind, placebo-controlled clinical trial. *Phytother Res*. 2009;23:1186-1189.
10. Rahbar A, Nabipour I. The hypolipidemic effect of *Citrullus colocynthis* on patients with hyperlipidemia. *Pak J Biol Sci*. 2010;13: 1202-1207.
11. Kumar S, Kumar D, Jusha M, Saroha K, Singh N, Vashishta B. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. *Acta Pharm*. 2008;58:215-220.
12. Jayaraman R, Shivakumar A, Anitha T, Joshi V, Palei N. Antidiabetic effect of petroleum ether extract of *Citrullus colocynthis* fruits against streptozotocin-induced hyperglycemic rats. *Rom J Biol Plant Biol*. 2009;4:127-134.
13. Najafi S, Sanadgol N, Nejad BS, Beiragi MA, Sanadgol E. Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) Schrad against *Staphylococcus aureus*. *J Med Plant*. 2010;4:2321-2325.
14. Salama HM. Alkaloids and flavonoids from the air dried aerial parts of *Citrullus colocynthis*. *J Med Plants Res*. 2012; 6:5150-5155.
15. Nickavar B, Kammalinejad M, Haj-Yahya M, Shafaghi B. Comparison of the free radical scavenging activity of six Iranian Achillea species. *Pharm Biol*. 2006;44:208-212.
16. Corda M, Giorgi O, Longoni B, Orlandi M, Biggio G. Decrease in the function of the γ-aminobutyric acid-coupled chloride channel produced by the repeated administration of pentylenetetrazol to rats. *J Neurochem*. 1990;55:1216-1221.
17. Mehrzadi S, Sadr S, Hosseinzadeh A, et al. Anticonvulsant activity of the ethanolic extract of *Punica granatum* L. seed. *Neurol Res*. 2015;37:470-475.
18. File SE, Pellow S. Intrinsic actions of the benzodiazepine receptor antagonist Ro 15-1788. *Psychopharmacology*. 1986;88:1-11.
19. Hosseinzadeh H, Parvardeh S. Anticonvulsant effects of thymoquinone, the major constituent of Nigella sativa seeds, in mice. *Phytomedicine*. 2004;11:56-64.
20. Kaminski RM, Witkin JM, Shippenberg TS. Pharmacological and genetic manipulation of kappa opioid receptors: effects on cocaine- and pentylenetetrazol-induced convulsions and seizure kindling. *Neuropharmacology*. 2007;52:895-903.
21. Lauretti GR, Ahmad I, Pleuvry B. The activity of opioid analgesics in seizure models utilizing *N*-methyl-DL-aspartic acid, kainic acid, bicuculline and pentylenetetrazole. *Neuropharmacology*. 1994;33:155-160.
22. Chopra R. *Chopra's Indigenous Drugs of India*. Calcutta, India: UN Dhur and Sons; 1958.
23. Upadhyay B, Roy S, Kumar A. Traditional uses of medicinal plants among the rural communities of Churu district in the Thar Desert, India. *J Ethnopharmacol*. 2007;113:387-399.
24. Qureshi R, Bhatti GR, Memon RA. Ethnomedicinal uses of herbs from northern part of Nara desert, Pakistan. *Pak J Bot*. 2010;42: 839-851.
25. Diwan F, Abdel Hassan I, Mohammed S. Effect of saponin on mortality and histopathological changes in mice. *East Mediterr Health J*. 2000;6:345-351.
26. Riazi K, Honar H, Homayoun H, et al. Sex and estrus cycle differences in the modulatory effects of morphine on seizure susceptibility in mice. *Epilepsia*. 2004;45:1035-1042.
27. Meena MC, Patni V. Isolation and identification of flavonoid quercitin from *Citrullus colocynthis* (Linn.) Schrad. *Asian J Exp Sci*. 2008;22:137-142.